Supporting information

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1.1 Reagents and Materials

HbA1c control samples of four levels of glycosylated hemoglobin were obtained from Kamiya Biomedical company (USA), and used without further purification. The lyophilized HbA1c samples are a haemolysate prepared from packed human erythrocytes, with stabilizers added to maintain haemoglobin in the reduced state for the accurate calibration of the HbA1c procedure. *N*-glycosylated pentapeptide (*N*-glycosylated-Val-His-Leu-Thr-Pro, purity by HPLC >97.5%) was purchased from Tocris bioscience (UK). Human HbA1c monoclonal antibody IgG (anti-HbA1c IgG) was supplied from Abnova (USA). The molecular wire was synthesized by following the methods from Tour and co-workers with some modifications, and was reported elsewhere.[1] Oligo(ethylene glycol) was synthesized according to the method reported.[2] 1,1'-di(aminomethyl)ferrocene was synthesized following the procedure from Ossola.[3] Reagent grade dipotassium orthophosphate, potassium dihydrogen orthophosphate, potassium chloride, sodium hydroxide, sodium chloride, sodium nitrite, hydrochloric acid, methanol and diethyl ether were purchased from Ajax Chemicals Pty Ltd. (Sydney, Australia). Potassium ferricyanide $K_4[Fe(CN)]_6$,

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 1,3-dicyclohexylcarbodiimide (DCC), ferrocenecarboxaldehyde, sodium cyanoborohydride, dimethylsulfoxide (DMSO), hemoglobin, bovine serum albumin (BSA), anti-biotin IgG from goat, and absolute ethanol were obtained from Sigma-Aldrich (Sydney, Australia). All reagents were used as received, and aqueous solutions were prepared with Milli-Q water (18 $M\Omega \cdot cm^{-1}$, Millipore, Sydney, Australia). Phosphate buffered saline (PBS) solutions were 0.137 M NaCl and 0.1 M K_2HPO_4/KH_2PO_4 and adjusted with NaOH or HCl solution to pH 7.3. Phosphate buffer solutions used in this work were 0.05 M KCl and 0.05 M K_2HPO_4/KH_2PO_4 and adjusted with NaOH or HCl solution to pH 7.0.

1.2 –Electrochemical measurements

All electrochemical measurements were performed with a BAS-100B electrochemical analyser (Bioanalytical System Inc., USA) and a conventional three-electrode system. GC electrodes (Bioanalytical Systems Inc., USA) were prepared from 3 mm-diameter rods embodied into epoxy resin and were used as working electrodes. Platinum foil and a Ag/AgCl (3.0 M NaCl) electrode were used as the counter and reference electrodes, respectively. All potential reported *versus* the Ag/AgCl reference electrode at room temperature. All cyclic voltammetry (CV) and square wave voltammetry (SWV) measurements were carried out in pH 7.0 phosphate buffer.

1.3 *In situ* modification of GC electrodes with mixed layer of MW/OEG

Commercial GC electrodes were hand-polished successively in 1.0, 0.3, and 0.05 μm

alumina slurries made from dry Buehler alumina mixed with Milli-Q water (18 MΩ. Cm^{-1}) on microcloth pads (Buehler, Lake Bluff, IL, USA). The electrodes were thoroughly rinsed with Milli-Q water and sonicated in Milli-Q water for 2 min. Before derivatization, the electrode was dried with a stream of argon. Surface derivatization of GC electrodes with mixed layer of MW/OEG was achieved by electrochemical reduction of *in situ*-generated binary aryl diazonium cations of MW and OEG in acidic aqueous solution**.** Specifically, a mixture of 5 mM MW which was firstly dissolved in a minimum amount of DMSO, and OEG (the molar ratio of MW to OEG was 1:50) was solubilized in 0.5 M HCl aqueous solution, and 5 mM of sodium nitrite was added to generate the aryl diazonium salts in the electrochemical cell (*in situ*), which would attach to the GC electrode surfaces immediately by cyclic voltammetry between 0.6 V and -1.1 V for two cycles at the scan rate of 100 mV s^{-1}.

1.4 Fabrication of the sensing interface

After surface modification with the mixed layer of MW/OEG, the GC electrode was ready for the fabrication of the sensing interface. This involves the step attachment of 1,1'-di(aminomethyl)ferrocene (FDMA) followed by *N*-glycosylated pentapeptide (GPP). Covalent attachment of FDMA to the carboxylic acid terminated mixed layer of MW/OEG was achieved by incubating the MW/OEG modified GC electrodes into absolute ethanol containing of 40 mM DCC and 5 mM FDMA for 6 h at room temperature. Any nonspecific adsorption of FDMA was removed by sonicating the surfaces in Milli-Q water for 2 min or continuous cycling between 0 V and 0.8 V in phosphate buffer until a stable electrochemistry was obtained. After the attachment of

FDMA, the GC electrode, now possessing distal amine groups, were immersed into 2 mM solution of GPP which has been prepared in phosphate buffer, pH 6.8 containing 20 mM of EDC and 10 mM of NHS for 4 h at 4 ^{o}C to attach the glycosylated pentapeptide to the free terminal amines on the surface bound FDMA. Then GPP terminated surface was incubated in 250 ng mL^{-1} human HbA1c monoclonal antibody IgG solution for 3 h at 4 ^{o}C.

1.5 Preparation of HbA1c control samples

Each lyophilized HbA1c control sample was reconstituted by adding 0.5 mL Milli-Q water, and the mixture was mixed gently for 10 min and stored at 4 ^{o}C as a stock solution. Based on the values detected by the instrument Hitachi 917 On board Lyse, the glycosylated haemoglobin levels were 4.5%, 8%, 12.1% and 15.1%, respectively, of total hemoglobin (glycosylated and non-glycosylated) concentration for each control sample. The total concentration of haemoglobin for four control samples is about 14.5 g dL^{-1}. The clinically recommended haemoglobin level is 11-16 g dL^{-1}. Samples with different glycosylated haemoglobin level were prepared by mixing control sample R1 (4.5%) and control sample R4 (15.1%) stock solutions in different ratio. To perform the competitive inhibition assay, samples with HbA1c analyte were preincubated with 2 µg mL^{-1} HbA1c monoclonal antibody for 30 min. Then 5 µL of mixture of HbA1c and HbA1c antibody were applied to the working area of GPP terminated GC electrode surfaces for 5 min followed by the electrochemical measurements.

1.6 Electrochemistry for the *in situ* modification of MW/OEG mixed layers on

GC electrodes

Fig. S1 (a) presents the cyclic voltammograms of a GC electrode modified with a mixed layer of MW/OEG. A significant broad peak was observed at about -0.8 V in the first cycle and disappeared in the second cycle, which indicates the reductive adsorption of MW/OEG molecules on the electrode surface. This grafted layer restricts further electrochemical reduction of the diazonium salt, and consequently, its own growth. A small peak at 0.2 V was observed in the first cycle, and its origin is not yet well understood although it also appears in other diazonium salts modification process. The MW/OEG mixed layers modified GC electrodes showed good blocking property in ferricyanide (1 mM; 0.05 M KCl; 0.05 M phosphate buffer; pH 7.0) (Fig. S1 a), suggesting the formation of organic layers on the electrode surfaces.

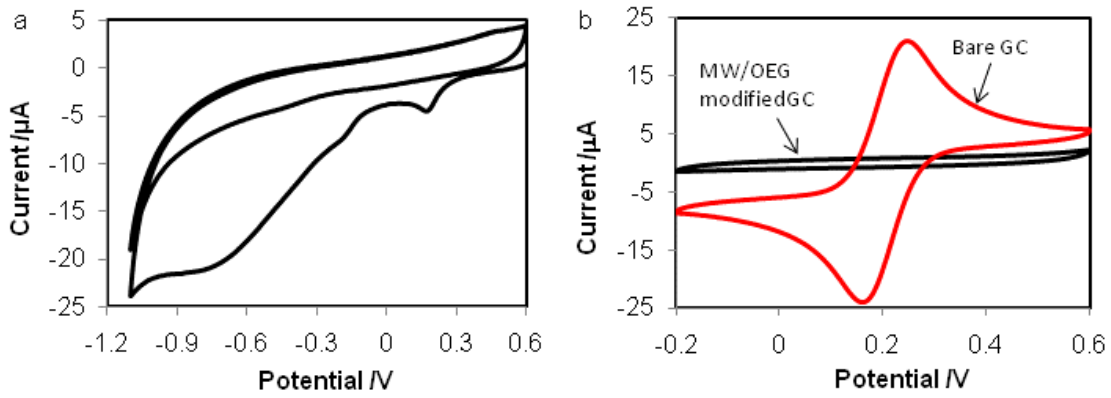

Fig. S1 CV for a bare GC electrode (a) in a mixture of 5 mM MW and OEG in molar ratio of 1:50 aryl diazonium salts obtained in acidic aqueous medium with 0.5 M HCl and 5 mM $NaNO_2$ as supporting electrolyte at the scan rate of 100 mV s^{-1} for the modification of MW/OEG layers; (b) before and after modification of MW/OEG layers in ferricyanide solution (1 mM; 0.05 M KCl; 0.05 M phosphate buffer; pH 7.0) at the scan rate of 100 mV s^{-1}.

1.7 Electrochemistry for the stepwise attachment of FDMA, *N*-glycosylated-VHLTP, and human HbA1c monoclonal antibody

Cyclic voltammogams measured in an aqueous solution of 0.05 M phosphate buffer (0.05 M KCl, pH 7.0) at a scan rate of 100 mV s^{-1} before and after the attachment of FDMA onto the MW/OEG layers modified GC electrode are shown in Fig. S2. The obvious redox peaks from ferrocene with $E_{1/2}$ of 340 mV and E_{FWHM} of 236 mV were observed, and during cyclic voltammetry the current did not show a detectable decrease with repeated cycling.

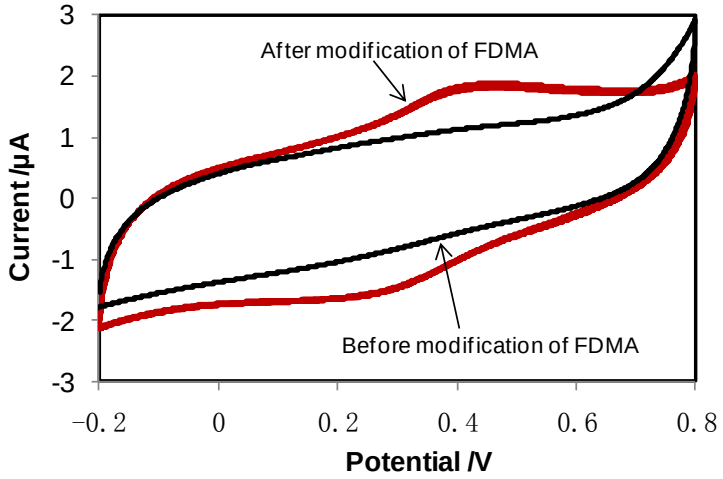

Fig. S2 CV of MW/OEG modified GC electrodes before and after attachment of FDMA.

1.8 Control on the selectivity of sensing interface

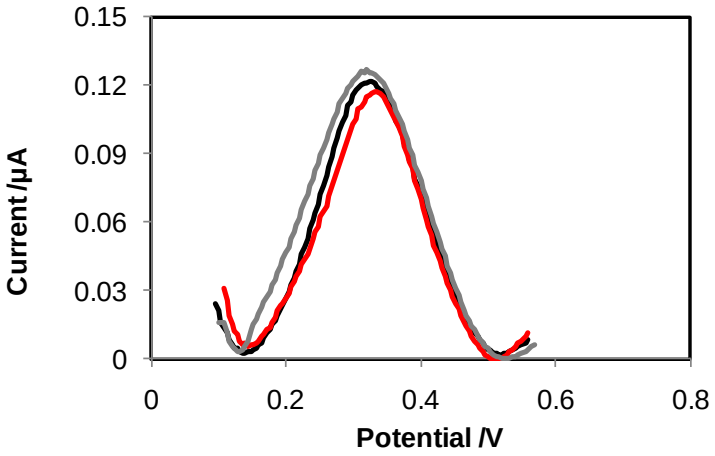

Fig. S3 SWV for FDMA modified GC electrodes after the attachment of GPP (grey line) followed by exposure to 10 μg mL^{-1} anti-biotin IgG (black line), or 10 μg mL^{-1} bovine serum albumin (red line).

1.9 Control on the affinity between HbA1c and anti-HbA1c IgG

As shown in Fig. S4 the current decreased to 36 %±6% (95% confidence, n=6), and 39 %±3% (95 % confidence, n=6) after the exposure of GPP modified GC sensing interfaces to 2 μg mL^{-1} human anti-HbA1c IgG which was premixed with 2 mM pentapeptide VHLTP, and 10 μg mL^{-1} haemoglobin for 30 min at 4 ^{o}C, respectively. This current decrease is almost the same as the current decrease observed for the exposure to pure anti-HbA1c IgG, but significantly higher than that for exposure to anti-HbA1c IgG containing HbA1c.

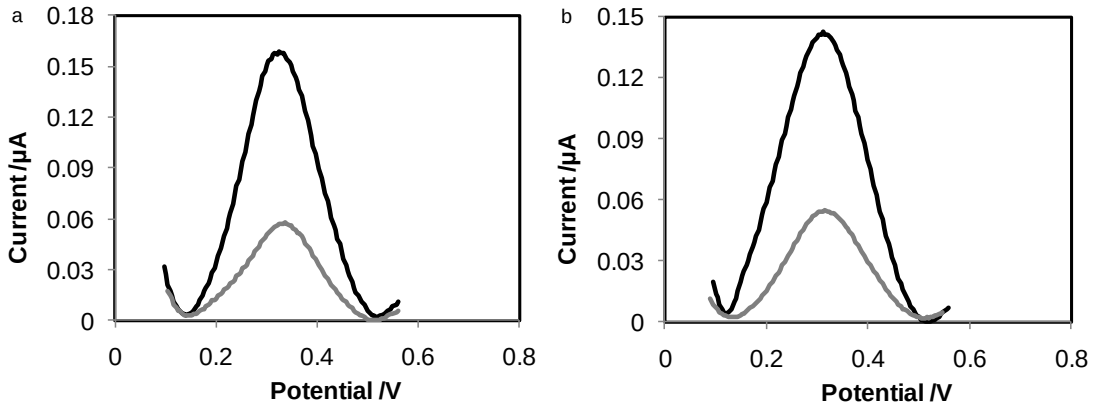

Fig. S4 Representative SWV for GPP modified GC sensing interfaces after incubation with 2 μg mL^{-1} human anti-HbA1c IgG which was premixed with (a) 2 mM pentapeptide VHLTP, and (b) 10 μg mL^{-1} haemoglobin, respectively for 30 min at 4 ^{o}C. Black line: GPP interface; grey line: GPP interface after exposure to a, and b solutions.

1.10 Optimization of the concentration of human HbA1c monoclonal antibody used in the competitive inhibition assay

The concentration of HbA1c monoclonal antibody used for the preincubated mixture of HbA1c and anti-HbA1c antibody is crucial for the success of the proposed competitive inhibition assay. Thus, four different concentrations of HbA1c monoclonal antibody IgG (0.5 μg mL^{-1}, 2 μg mL^{-1}, 5 μg mL^{-1}, and 10 μg mL^{-1}) were tested for this assay. Four HbA1c control samples with the glycosylated haemoglobin (HbA1c) levels of 5.0%, 7.9%, 11% and 13.5%, respectively, of total hemoglobin (glycosylated and non-glycosylated) concentration were used. It is found that when the concentration of anti-HbA1c antibody is 2 μg mL^{-1} , the assay gave good linear relationship between the relative current and the concentration of analyte HbA1c. In addition, the slope of the calibration curve is higher when the HbA1c antibody

concentration is 2 μg mL^{-1}, indicating higher sensitivity (Fig. S5). With other antibody concentrations, good linear relationship between the relative current and the concentration of analyte HbA1c was also obtained, but the slope of the calibration curves were lower, suggesting lower sensitivity. Thus, the conclusion can be drawn that 2 μg mL^{-1} HbA1c monoclonal antibody is the best concentration to mix with HbA1c analyte under the current working conditions.

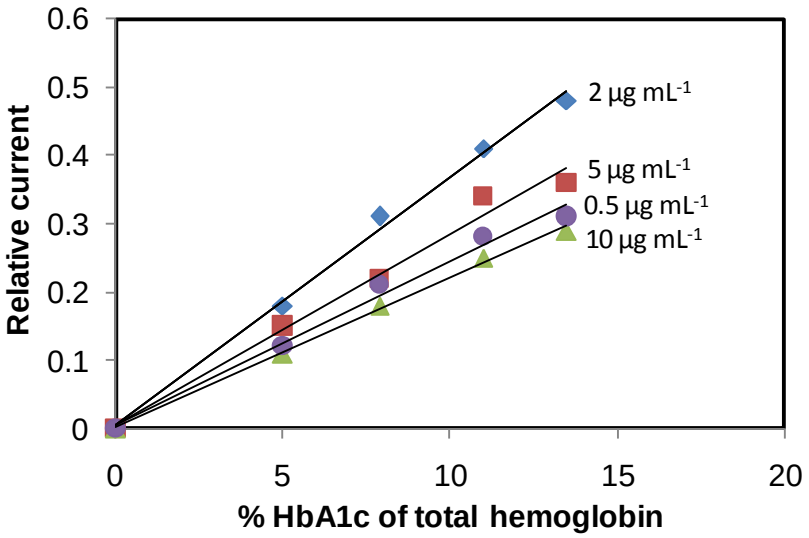

Fig. S5 Calibration curve for HbA1c measured with the competitive inhibition assay, Four HbA1c control samples were premixed with different concentration of anti-HbA1c monoclonal IgG for 30 min at 4 ^{o}C, respectively before applying to the GPP modified sensing interface for 5 min.

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2. J. L. Bahr, J. Yang, D. V. Kosynkin, M. J. Bronikowski, R. E. Smalley and J. M. Tour, *J. Am. Chem. Soc.*, 2001, **123**, 6536-6542.
3. F. Ossola, P. Tomasin, F. Benetollo, E. Foresti and P. A. Vigato, *Inorg. Chim. Acta*, 2003, **353**, 292-300.